Effect of pH and Other Conditions on the Fractionation of Gelatins in Carboxymethylcellulose Columns

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Commercial gelatins from limed calf skin could be divided arbitrarily into two fractions by varying the pH in the fractionation on carboxymethylcellulose columns.

A modification of the method of fractionation on carboxymethylcellulose for preparative work is described.

The purpose of this report is to describe in detail the effect of pH on the fractionation of gelatins on carboxymethylcellulose (CM) columns by a method which has been developed earlier[1,2,3]. To permit further analysis of the fractions, we have modified the conditions for their isolation on a preparative scale.

Experimental

Samples. Two gelatin samples, both prepared from lime-treated materials, were used in the study. The gelatin referred to in Fig. 1 was designated VII in our earlier publication[2]. It has a Bloom value of 244 and a pI of 4.95, and was obtained from Kind & Knox Gelatin Company, Camden, N.J., U.S.A., through the courtesy of Dr. E. M. Marks.

The sample to which Fig. 2 refers (purchased from E. Merck AG, Darmstadt, Germany, and reportedly prepared from »cartilages and good bones») was designated I in our previous report. Before the fractionation this sample was allowed to become infected and liquefied.

The ion-exchange material used was Whatman carboxymethylcellulose powder, labelled CM-70. The separations obtained differed somewhat from lot to lot of the powder.

Analytical chromatography. In the study of the pH effect, the procedure described earlier[2], was applied

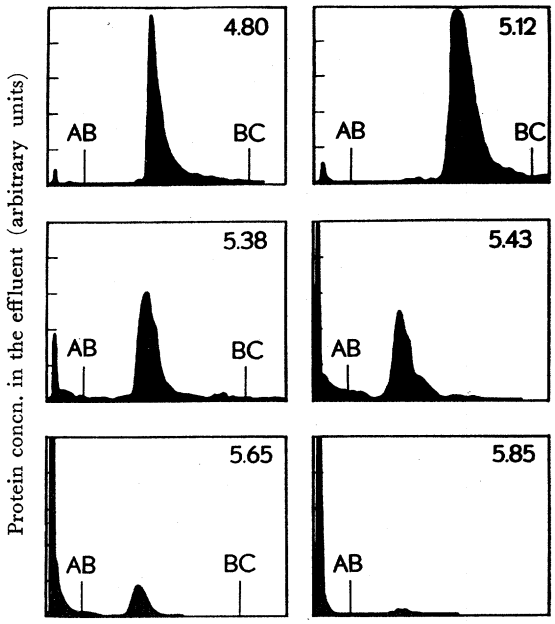

Fig. 1. Fractionation of the same gelatin sample on a CM-cellulose column by solutions of different pH. In the range from the beginning to AB (usually 20 fractions, 40 ml) the column was eluted with a 0.01 *M* sodium acetate buffer of the indicated pH. In the range AB-BC (usually 80 fractions, 160 ml) the elution was continued with an exponential salt strength gradient (0.86 % sodium chloride was passed from the reservoir into the closed, magnetically stirred mixing chamber of 100 ml). From BC onwards a gradient with 2.5 % NaCl was superimposed.

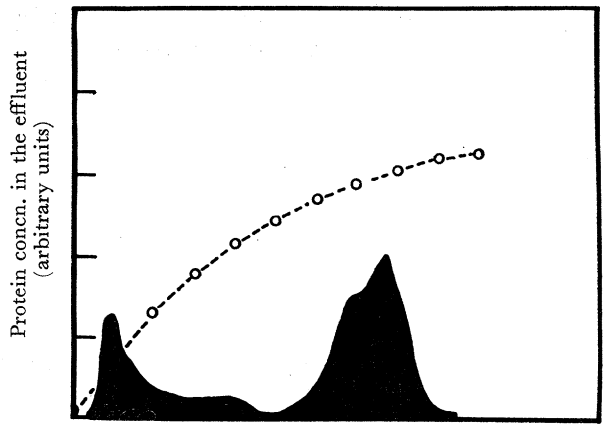

Fig. 2. Fractionation of an infected gelatin sample which contained 190 mg of commercial gelatin from limed materials. The CM-cellulose bed, 2 × 30 cm, was pretreated with 0.01 *M*, pH 4.80, sodium acetate buffer. The temperature in the water jacket was + 38°C during the run. Mixing volume 500 ml; total volume of tubing 50 ml. Elution rate 180 ml/hr; fraction volume 15 ml.

After 350 ml of 0.01 *M*, pH 4.80, sodium acetate buffer had been passed through, the gradient was applied (0.86 % NaCl into the mentioned buffer). The dotted line shows the ionic strength gradient, the last value being 0.13. The total volume of the effluent from the beginning to the end of the gradient was 1000 ml.

except that sodium chloride solutions were used instead of magnesium chloride solutions. The column was treated with a 0.01 *M* acetate buffer of the desired pH until the effluent had the same pH. When the gelatin fraction emerged, the pH of the eluate dropped about 1.2 pH units, but increased afterwards to a slightly more acid level.

Preparative chromatography. The dimensions of the CM-cellulose bed in a water-jacketed column which was maintained at + 38°C were 2 × 30 cm. The CM-cellulose was allowed to swell in 0.01 *M*, pH 4.80, sodium acetate buffer in a cold room for at least one night. It was then poured in small amounts as a slurry into the column and packed with nitrogen gas under pressure. The column was washed with deaerated buffer until the pH of the effluent was 4.80. The sample (50—200 mg), which had been dialyzed against a 0.01 *M*, pH 4.70, sodium acetate buffer, was then added to column and the run begun.

The fluid in the mixing vessel was kept under liquid paraffin and deaerated solvents and capillary tubings were used throughout. The flow rate was 80—100 ml/h. A water-jacketed siphon was constructed for the collection of 10-ml fractions. The column was first eluted with the 0.01 *M*, pH 4.80, sodium acetate buffer (250 ml plus the sample volume). The exponential gradients were produced by feeding the salt solution into a closed mixing vessel, which contained originally the sodium acetate buffer only. The first gradient (3000 ml) was run into the closed mixing chamber (mixture volume 1350 ml) from a reservoir containing a 0.86 % solution of sodium chloride in the mentioned acetate buffer. The second gradient was carried out with a 10 % solution of sodium chloride run into the same buffer. This modification was useful in the study of the subfractions of only slightly degraded collagens[5,6].

The protein in the fractions was determined by a modified biuret reaction according to Lowry *et al.*[4], as a rule from 0.3 ml of the eluate.

Results and Discussion

Fig. 1 shows the analytical fractionation pattern of a commercial gelatin (from limed calf skin) at different pH values. At pH 4.80 the gelatin is at first almost completely bound to the column, but eluted later as a single, although heterogeneous, peak. When the fractionation is carried out at a higher pH, a part of the material is not retained. At a pH over 5.8 the material runs straight through the column, except for a small part that is retained on the ion-exchange material even at pH 7.0. The various gelatin molecules can be thus divided into two fractions in an arbitary way by choosing a suitable pH. The nature of the two small anomalous fractions (one not bound at pH 4.8 and one retained even at pH 7) was not studied. They may be non-collagenous contaminants such as carbohydrates, or other proteins.

Fig. 2 shows the preparative fractionation of 190 mg of a commercial gelatin at pH

4.80. The original preparation yielded normally a pattern like that shown in Fig. 1, but the sample had been allowed to deteriorate under the action of air-borne bacteria at room temperature. The sample was divided into fractions which were partly released from the column already by a dilute salt solution.

This work is a part of a program supported by U.S. Department of Agriculture, Foreign Research and Technical Programs Division.

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Received January 13, 1964.